VOLATILE SELENIUM IN HIGHER PLANTS

THE PRODUCTION OF DIMETHYL SELENIDE IN CABBAGE LEAVES BY ENZYMATIC CLEAVAGE OF Se-METHYL SELENOMETHIONINE SELENONIUM SALT

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SUMMARY

The volatile selenium compound produced by cabbage (Brassica oleracea var. Capitata) when cultured on media containing either selenite or selenate is dimethyl selenide, (CH₃)₂ Se. The dimethyl selenide arises from enzymatic cleavage of a Se-methyl selenomethionine selenonium compound.

INTRODUCTION

The biological production of volatile selenium compounds has been demonstrated by animals, micro-organisms, and higher plants. Within the first two groups, the compound has been widely identified as dimethyl selenide. Identification of volatile selenium from higher plants, however, has been limited to one of several compounds produced by a selenium accumulator (Astragalus racemosus), namely, dimethyl diselenide. Dimethyl selenide was not detected in volatile material from this species.

METHODS AND MATERIALS

Plants were cultured in a greenhouse over standard nutrient solutions with addition of 37 μM of either K₂SeO₃ or K₂SeO₄, labeled with Se⁷⁵. The compo-
sition of the standard nutrient solution was as follows (in μM): nitrogen (NO₃⁻) 14,000; potassium 7,000; calcium 4,000; magnesium 1,000; sulfur 1,000; phosphorus 1,000; chlorine 280; sodium 10; boron 25; iron (as Fe-EDTA) 90; manganese 5; zinc 2; copper 0.5; and molybdenum 0.5. Leaf blades were harvested, rinsed in distilled water, and homogenized in buffer solutions at a specified pH. In general, a 1:10 proportion of fresh leaf blades to cold buffer was used. Aliquots of the resulting suspension were taken for determination of Se⁷⁵ activity using a welltype NaI-Tl scintillation counter. Measured volumes of the homogenates (usually 200 ml) were placed in 500-ml suction flasks connected to tubes containing 2 to 5 g of activated charcoal granules. Air used to aerate the homogenates was cleaned by previously passing it through tubes containing activated charcoal and non-absorbent cotton. This clean air was allowed to flow through the homogenate and after a definite period of time the Se⁷⁵ activity trapped on the charcoal from the outgoing airstream was determined. Proportions of volatile selenium were calculated as follows:

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\text{Volatile Se (\%)} = \frac{\text{Total Se}^{75} \text{ on charcoal (counts/min)}}{\text{Total Se}^{75} \text{ in homogenate (counts/min)}} \times 100
\]

**Plant species**

Preliminary trials were run to permit selection of a plant species which would produce the largest yield of volatile selenium under the experimental conditions. Five cruciferous species, cabbage (Brassica oleracea var. Capitata), Chinese cabbage (Brassica pekinensis), radish (Raphanus sativus), turnip (Brassica rapa), mustard (Brassica juncea), and a composite, sunflower (Helianthus annuus, dwarf variety) were evaluated for growth rate, accumulation of selenium and rate of production of volatile selenium.

With all species, selenium concentrations in the leaves generally increased with time of culture. The percentage of volatile selenium appeared to be independent of leaf Se concentration, ranging from less than 0.05 to 0.30 percent of the leaf concentrations at the extremes. Cabbage (B. oleracea var. Capitata) was selected because of ease of culture and high yield of volatile selenium.

**Effect of the oxidation state of selenium supplied**

Experiments were performed to determine the effect of the oxidation state of selenium (+4 in selenite and +6 in selenate) on the production of volatile selenium by cabbage leaf homogenates.

Two sets of cabbage plants were cultured in 40-liter tanks of nutrient solution, one of which contained 39 μM K₂Se⁷⁶O₃, and the other 39 μM K₂Se⁷⁸O₄. The plants were 33 days old at the time of transfer to the selenium solutions and the first harvest was made 17 days thereafter.

Fresh blades were sampled from the two sets of harvested plants, and 100 g of each were homogenized in 0.1 M phosphate buffer at pH 7.2. The concentration of the homogenates was 0.181 g fresh leaves/ml and the pH was 6.70